GEORGIA INSTITUTE OF TECHNOLOGY — OFFICE OF CONTRACT ADMINISTRATION

PROJECT ADMINISTRATION DATA SHEET

[X] ORIGINAL [ ] REVISION NO. ____

Project No. G 33-G06 DATE 9-4-81

Project Director: NAI-TENG YU School/Lab Chemistry

Sponsor: DHHS/PHS/NIH - National Institute of General Medical Sciences

Type Agreement: Grant No. 5-R01-GM 18894-11

Award Period: From 9-1-81 To 8-31-82 (Performance) 11-30-82 (Reports)

Sponsor Amount: $ 74,619 Contracted through:

Cost Sharing: $ 3,927 (G33-371) ~~GTRC~~/GIT

Title: Laser-excited Raman Spectroscopy of Biopolymers

ADMINISTRATIVE DATA OCA Contact Don S. Hasty

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Defense Priority Rating: N/A

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Security Classification: N/A

RESTRICTIONS

See Attached NIH Supplemental Information Sheet for Additional Requirements.

Travel: Foreign travel must have prior approval - Contact OCA in each case. Domestic travel requires sponsor approval where total will exceed greater of $500 or 125% of approved proposal budget category.

Equipment: Title vests with N/A - None proposed -

COMMENTS:

Fellow on project To G33-G05 (10 yr)

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FORM OCA 4:781

GEORGIA INSTITUTE OF TECHNOLOGY OFFICE OF CONTRACT ADMINISTRATION

SPONSORED PROJECT TERMINATION SHEET

Date 7/12/83

Project Title: Laser-Excited Raman Spectroscopy of Biopolymers

Project No: G-33-G06

Project Director: Dr. Nai-Teng-Yu

Sponsor: DHEW/PHS/NIH - National Institute of General Medical Sciences

Effective Termination Date: 8/31/82

Clearance of Accounting Charges: 11/30/82

Grant/Contract Closeout Actions Remaining:

NONE

- ☐ **Final Invoice and Closing Documents**
- ☐ **Final Fiscal Report**
- ☐ Final Report of Inventions
- ☐ Govt. Property Inventory & Related Certificate
- ☐ Classified Material Certificate
- ☐ Other _____

NOTE: Follow-on project (11 year) - G-33-C07

Assigned to: Chemistry (School/~~Laboratory~~)

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FORM OCA 10:781

C. Progress Report/Preliminary Studies

(i) Period: September 1, 1978 - August 21, 1981

(ii) Professional Personnel who have worked on the project:

Yu, Nai-Teng, Professor (Principal Investigator),	09/01/78 - present,	50%
Srivastava, R. B., Research Scientist,	12/18/78 - 06/31/80,	100%
	07/01/80 - present,	15%
Pace, C. Research Associate,	12/01/78 - 06/30/81	
Tsubaki, M., Research Technician,	04/01/79 - 03/20/81,	100%
Mackin, H. C., Research Technician,	06/15/81 - present,	100%

(iii) Previous Application's Specific Aims: In previous application we proposed to achieve the following specific aims: (1) To develop "resonance Raman excitation profile" techniques for biological and biochemical applications, employing tunable lasers covering the UV and visible regions; (2) To elucidate the structural basis of hemoprotein functions; (3) To obtain Raman evidence for IHP-induced cleavage of proximal histidine to iron bond in nitrosyl HbA; (4) To establish via resonance Raman excitation profiles whether a mercaptide is co-ordinated to heme in cytochrome P-450; (5) To determine the extent of Jahn-Teller distortion in cytochrome c and cytochrome b_{562}; (6) To bring out low-frequency axial ligand modes of metalloporphyrins and hemoproteins; (7) To further develop resonance CARS and resonance RIKES technique for application to highly fluorescent biological samples; (8) To extract structural information from nucleic acid-carcinogen complexes, using CARS and RIKES methods.

(iv) Summary of Progress and Important Findings:

(a) The most important step taken during this period is the development and the successful operation of a highly sensitive multichannel Raman system which has already begun to produce exciting new results. This system is specifically designed for heme proteins research. It has high throughput, unvignetted image on detector, excellent stray-light rejection and optimum bandpass/resolution, and is equipped with two most advanced multichannel detectors (PAR Model 1254 cooled SIT and PAR model 1420 intensified SPD). As we have demonstrated in our publications, it is an ideal device for the systematical search of axial ligand-associated vibrations in hemoproteins and synthetic model hemes, for the measurement of resonance Raman excitation profiles and for the recording of time-resolved resonance Raman spectra of short-lived (msec-picosecond) transient species.

Comparison of the spectral responses of the two detectors is shown in Fig. 1. The intensified reticon (PAR 1420) is particularly sensitive (20% Q.E.) for UV resonance Raman spectroscopy in the 200-400 nm region (specific aim no. 1 has been achieved).

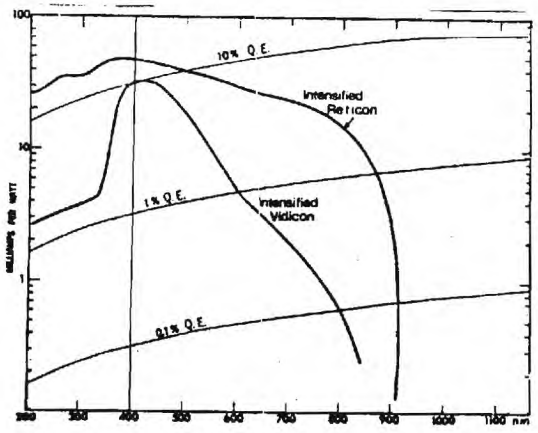


Figure 1

(b) To understand the structural basis for protein modulation of heme reactivity we have made major efforts in the resonance Raman enhancement of axial ligand vibrations in hemoproteins and model metalloporphyrins. Important identification/assignments include: (1) ν(O-O) and ν(Co-O) stretching vibrations in oxyCoMb/oxyCoHb (PNAS, 78, 3581 (1981)), in Co "picket fence" (N-MeIm)(O_2) (JACS, in press); (2) ν(Fe-CO) stretch, δ(Fe-C-O) bend and ν(C-O) stretch in HbCO/MbCO (Biochemistry, in press), in Fe(II)TPP(N-MeIM)(CO) (unpublished); (3) δ(N=N=N) bend and ν(N=N=N) antisym. stretch in MnMbN$_3$ (Biochemistry, 19, 4647 (1980));

(4) ν(Fe-N) stretch, δ(N=N=N) bend and ν(N=N=N) antisym. stretch in Fe(III)Mb-N_3 (Biochemistry, 20, 946 (1981)); (5) ν(Fe-NO) stretch and ν(N-O) stretch in HbNO/MbNO (Biochemistry, in press); (6) ν(Fe-CN) stretch in cyanomet Hb (FEBS, to be submitted); (7) ν(Fe-S) in cytochrome P-450$_{scc}$; (8) ν(Mn-N_εHis). These identifications are important for future research aiming at understanding the exact nature of protein-heme interactions. Our sensitive multichannel Raman system has greatly facilitated this often frustrating, but worthwhile adventure (specific aims no. 2, 4, & 6).

(c) A low frequency mode at 301 cm^{-1} in the spectrum of HbNO appears to be a good candidate for the ν[Fe-N_ε(His F8)] stretch because it reduces intensity by one half when IHP binds human nitrosyl HbA. We are in the process of obtaining further evidence for the assignment from nitrosyl Fe "picket fence" porphyrin complexes. In contrary to the work of Stong et al. (1980) we have not observed the ν(Fe-NO) stretch at 592 cm^{-1} from the pentacoordinated NO-heme complex in the α subunits. The ν(Fe-NO) stretch from hexacoordinated NO-heme complex was located at 551 cm^{-1} (Biochemistry, in press) (specific aim no. 3).

(d) We compared the excitation profiles of a depolarized porphyrin ring mode at ~750 cm^{-1} among cytochrome c, cytochrome b_{562} and Ni etioporphyrin. The effect of a Jahn-Teller distortion observed in the 750 cm^{-1} profile of Ni etioporphyrin (J. Chem. Phys. 66, 3387 (1977)) was shown to be absent in cytochrome c, but weakly presence in cytochrome b_{562} (J. Raman Spectrosc. 11, 20 (1981)) (See specific aim no. 5).

(e) Interactions of Co(III) with adenosine-5'-triphosphate in the presence of 1,10-phenanthroline and superoxide were studied by laser Raman scattering (J. Raman Spectrosc. 11, 150 (1981)). Striking intensity enhancement of two adenine ring vibrations at 734 and 1430 cm^{-1} was observed upon formation of the complex Co(III)-(phen)-ATP-O_2^-. These two lines are resonance-enhanced with a charge-transfer transition between adenine and Co(III) in the 300-400 nm region.

(f) We reported resonance Raman studies of cytochrome c at acidic pH values and the effect of anions on the Raman spectrum. The "core-expansion" correlation first proposed by us was used to argue the existence of an in-plane spin iron in a heme with two weak field axial ligands (water). Raman spectra of acid cytochrome c excited near 620 nm was presented (Biochemistry, 8, 1656 (1979)) and the nature of the electronic transition responsible for the 620 nm absorption has been discussed.

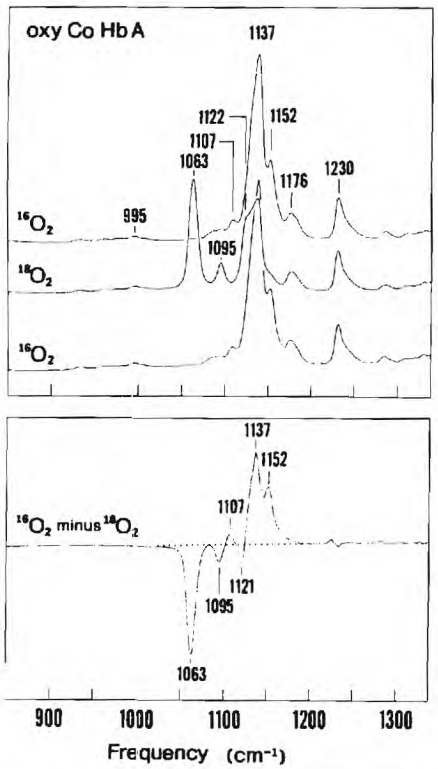
(v) Changes in the Project's Specific Aims During the Previous Project Period: The overall objectives of the entire project since 1971 have been: (a) To develop the techniques and procedures necessary to obtain and interpret the Raman spectra of biopolymers; (b) To derive significant structural information of proteins and hemeproteins not obtainable by other research techniques, and (c) To correlate the structure-function relationship of biomolecules. Under these broad objectives, our specific aims in each period change according to the specific promising techniques developed such as the advent of certain detectors or new laser technology. During the previous period, we have accomplished all the specific aims (no. 1-6) except those involving CARS and RIKES (no. 7 & 8). We decided not to emphasize these two nonlinear techniques because they are not as promising as multichannel Raman techniques for hemoprotein applications. With our limited equipment funds and manpower, it is not possible for us to develop both multichannel technique and nonlinear CARS and RIKES.

(vi) Detailed Progress Report: The work performed during the past three years has lead to over 22 manuscripts, of which 16 published and 6 in press. The main scientific findings and accomplishment may be described as follows:

(a) Structural Implication of Splittings of Bound O-O Stretching Vibration in OxyCoMb/OxyCoHb (with Tsubaki, PNAS, 78, 3581 (1978)).

The frequency of bound O-O stretching vibration in oxy hemoproteins has been the focus of considerable controversy and remained as a puzzle in biochemical literatures. Resonance Raman spectroscopy appears to have provided the necessary information for solving the problem of long standing.

This vibrational frequency was first reported by Caughey and coworkers using infrared spectroscopy (Barlow et al., 1973; Maxwell et al., 1974; Maxwell and Caughey, 1974) at 1107 (oxy HbA), 1103 (oxyMb) and 1105 cm^{-1} (oxy Co-deuteroporphyrin IX-substituted HbA). These assignments were questioned by Collman et al. (1974 & 1975) who reported the ν(O-O) stretch at 1385 cm^{-1} in oxy Fe(II) "picket fence" porphyrin, a myglobin model heme. Later, the 1385 cm^{-1} infrared band was proven to be artifactual (Collman et al., 1976). Collman et al. (1976) have now located the ν(O-O) frequency at ~1150-1160 cm^{-1} in the oxygen adducts of Fe and Co "picket fence" porphyrins. However, these frequencies are still quite different from the values for oxyHb/Mb. The picture was further complicated by the observation of an IR band at 1156 cm^{-1} in oxyHbA (Alben et al., 1978). The splitting of the ν(O-O) mode into two IR bands at 1107 and 1156 cm^{-1} in oxyHbA was interpreted as due to Fermi resonance with the first overtone of the $\nu(Fe-O_2)$ stretch at ~570 cm^{-1} observed by Brunner (1974) using resonance Raman spectroscopy. In our studies we detected three isotope-sensitive lines at 1103(1107), 1137(1137) and 1153(1152) cm^{-1} in the resonance Raman spectra of oxyCoMb (or oxyCoHbA). It now turns out that the kind of Fermi resonance suggested by Alben et al. (1978) may not be operative in both Fe and Co systems. The first two frequencies arise from resonance interaction (a vibrational perturbation involving two fundamentals) between a ν(O-O) mode at ~1122 cm^{-1} and an accidentally degenerate porphyrin ring mode at 1123(1121) cm^{-1}, whereas the third one represents an "unperturbed" ν(O-O) vibration from a different conformer. The ν(Co-O) stretch was detected at ~538 cm^{-1} which is considerably lower than the $\nu(Fe-O_2)$ frequency at ~570 cm^{-1} in oxyFeMb and oxyFeHbA. The Co-O bond is longer and weaker than the Fe-O bond.

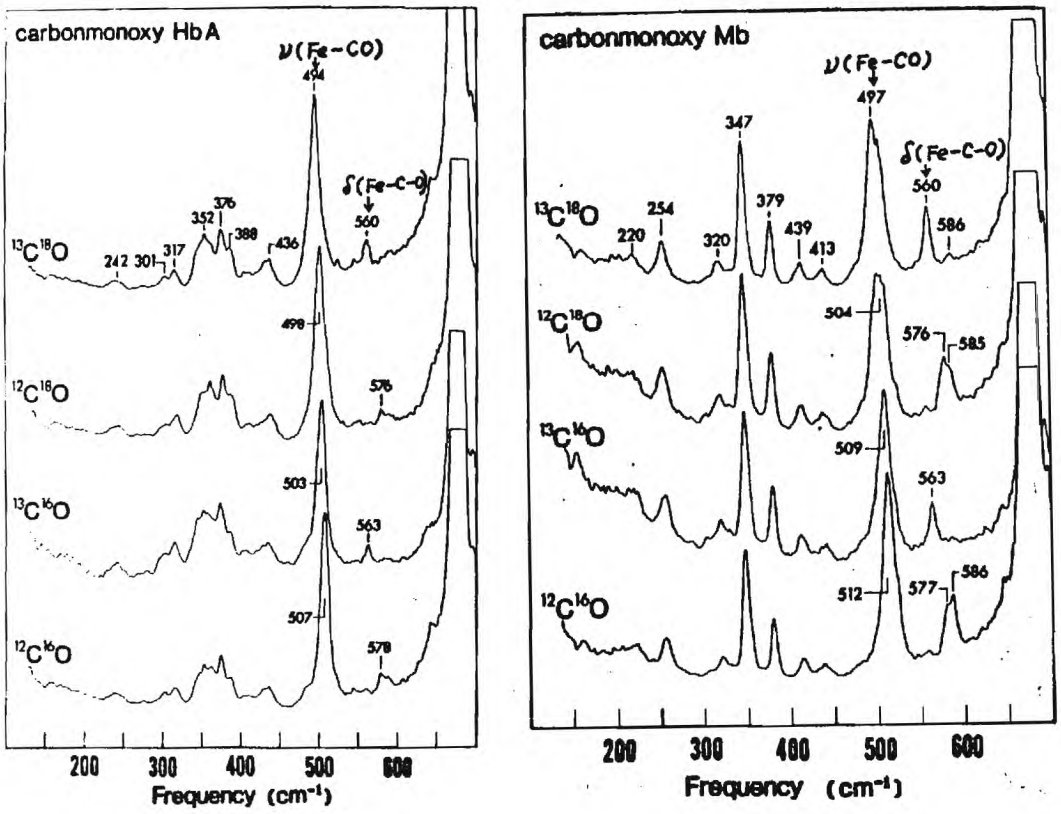


Resonance Raman enhancement of both ν(O-O) and ν(Co-O) indicates the existence of a charge-transfer transition underlying the Soret band, which has been assigned as $\pi^*(\pi^*O_2/d_{xz}) \rightarrow \sigma^*(d_{z^2}Co/\pi^*_g)$. The bound O_2 in a bent end-on configuration may have two allowed orientations which differ in the extent of $sp^2(N_\varepsilon) \rightarrow \pi^*(O_2)$ donation from distal histidine. The observed ν(O-O) mode at ~1152 cm^{-1} in oxyCoMb and oxyCoHbA is close to the modes of dioxygen adducts of Fe or Co "picket fence" porphyrin complexes (~1150-1160 cm^{-1}) in which there is no donation to bound dioxygen from the distal site. Fig. 2 shows resonance Raman spectra of oxyCoHbA (upper) and difference spectrum ($^{16}O_2$ minus $^{18}O_2$) (lower) in the 900 to 1300 cm^{-1} region.

(b) Detection of Fe-CO Stretching and Fe-C-O Bending Vibrations in Carbonmonoxy Hb and Mb (with Tsubaki and Srivastava, Biochemistry, in press).

Identification of ν(Fe-CO) stretch in hemoproteins by resonance Raman has been extremely difficult because carbon monoxide dissociates from heme easily upon illumination of laser light. The quantum yield for photodissociation of carbonmonoxy Mb is as high as 1.0 at 21°C (Stanford et al., 1980). However, with our sensitive multichannel Raman system and proper choice of exciting wavelength, we have succeeded in obtaining high-quality resonance Raman spectra of carbonomonoxy Hb and Mb (Fig. 3) which contain practically no contribution from photolyzed deoxy species. With excitation

Figure 3



at 406.7 nm we observed lines at 507(512), 578(577) and 1951(1944) cm^{-1} sensitive to CO isotope substitution. On the basis of a linear Fe-C-O configuration (tilted away from the heme normal by ~13°) as revealed by the X-ray crystallographic studies of human carbonmonoxy HbA (Baldwin, 1980), the pattern of observed isotope ($^{13}C^{16}O$, $^{12}C^{18}O$, $^{13}C^{18}O$) shifts and normal coordinate calculations permit us to establish that the most intense line at 507(512) cm^{-1} is the ν(Fe-CO) stretching and the weaker one at 578(577) cm^{-1} is a δ(Fe-C-O) bending mode. The frequency at 1951 (1944) cm^{-1} is the ν(C-O) vibration in HbCO (MbCO).

Careful examination of the Fe-CO stretching mode at 507 cm^{-1} in carbonmonoxy HbA and Hb Kansas both with and without IHP reveals no changes in either frequency nor intensity. However, resonance Raman spectrum of carbonmonoxy carp Hb exhibits a broadening of the Fe-CO stretching line on the low energy side upon switching the quaternary structure from R- to T-form, suggesting the presence of a new conformer with a weaker Fe-CO bond or a somewhat different tilt angle between the Fe-C-O group and

the heme normal.

Both ν(Fe-CO) stretching and δ(Fe-C-O) bending are not detectable by infrared spectroscopy or other techniques. This is clearly an important development in hemoprotein research. It is now possible to employ carbon monoxide as a visible ligand to directly probe the heme reactivity and distal side nonbonding steric effects.

(c) Nitric Oxide Bonding in Nitrosyl HbA and Mb (with Tsubaki, Biochemistry, in press).

With excitation at 406.7 nm we detected the resonance Raman enhancement of bound ν(N-O) stretch at 1623 cm^{-1} in No-HbA and No-Mb, as well as the ν(Fe-NO) stretch at 551 cm^{-1}. The latter frequency can also be observable with the Q-band excitation, a phenomenon similar to the ν(Fe-O) and ν(Fe-CO) stretches in oxy- and carbonmonoxy hemoproteins. It appears that these iron-ligand vibrations may be resonance-enhanced via porphyrin $\pi \rightarrow \pi^*$ transitions. Upon addition of IHP at pH 6.0, the ν(Fe-NO) stretch at 551 cm^{-1} and a low frequency mode at 301 cm^{-1} exhibit an intensity decrease by one-half. We observed no resonance Raman lines attributable to the ν(Fe-NO) stretch or ν(N-O) stretch from the pentacoordinated NO-heme complex in the α subunits.

(d) Novel Observation: Resonance Raman Enhancement of Non-Totally Symmetric Internal Azide Vibrations in Mn(III)-Mb-N_3 Complex (with Tsubaki, Biochemistry, 19, 4647 (1980)).

We reported the first observation of resonance-enhanced non-totally symmetric bound ligand modes in hemoproteins. In contrast, Wright et al. (1979) observed only totally symmetric internal pyridine vibrational modes via resonance with the Fe(d_π) $\rightarrow$ pyridine(π^*) transition.

The enhancement of bound azide vibrations at 650 cm^{-1} [depolarized (dp), bending] and 2039 cm^{-1} (dp, antisymmetric stretch) upon excitation at ~400-460 nm indicates the existence of a new charge-transfer transition in Mn(III)Mb-azide complex. Interestingly, the Mn(III)-N_3 stretch is not enhanced. The resonance Raman spectra of Mn(III)Mb-azide between 150 and 300 cm^{-1} differ dramatically from those of Fe(III)Mb-azide excited in the 640 nm charge-transfer band or near the Soret band. There are lines at 170 and 282 cm^{-1} (both polarized) in the Mn(III)Mb-azide spectra which exhibit extremely large resonance enhancements and are unshifted by $^{15}N_3$ isotope substitution. These two lines, having no analogue in other heme protein spectra, was tentatively assigned to the out-of-plane porphyrin ring vibrations. The lack of enhancement of the Mn(III)-N_3 stretch leads us to the assignment of azide (π) $\rightarrow$ porphyrin (π^*) charge-transfer transition.

(e) Temperature Dependence of Resonance Raman Spectra of Metmyoglobin and Methemoglobin (with Tsubaki & Srivastava, Biochemistry, 20, 946 (1981)).

In this study, we have solved the controversial problem on the assignment of ν(Fe-N_3) stretch in metmyoglobin and methemoglobin. These complexes has an absorption band at ~640 nm, which has been assigned to an x,y-polarized high-spin charge-transfer transition (Eaton and Hochstrasser, 1968). When azide metmyoglobin is converted completely to the low-spin form (at 77°C), the absorption spectrum still shows a weak broad band centered at ~650 nm, which was suggested by Eaton & Hochstrasser (1968) as a charge-transfer band (Z polarized) corresponding to a transition of the low-spin form of metmyoglobin azide. By excitation into the 640-nm absorption region, Asher et al. (1977) observed the selective enhancement

of a Raman mode at 413 cm^{-1} in methemoglobin azide and assigned it to the Fe(III)-azide stretch. Later, Desbois et al. (1979) assigned a 570 cm^{-1} mode (excited near Soret band) in metmyoglobin azide to the Fe(III)-azide stretch. To explain the discrepancy between these two assignments, Asher & Schuster (1970) suggested that the 413 cm^{-1} mode was from high-spin and the 570 cm^{-1} mode from the low-spin form. However, we have demonstrated that with decreasing temperature the intensity of the 413 cm^{-1} mode increases in spite of the decrease in high-spin component (hence the absorption at ~640 nm). This is taken as a clear evidence that the 413 cm^{-1} mode derives its intensity from the weak low-spin charge-transfer band underlying the stronger high-spin charge transfer band at ~640 nm. Our temperature studies, isotope substitution, depolarization measurements, and normal coordinate analysis allow us to establish that the 413 and 570 cm^{-1} modes are Fe(III)-azide stretch and azide internal bending, respectively, both in low-spin state. In addition, we show the enhancement of bound azide antisymmetric stretch from both high- and low-spin forms upon excitation at 406.7 nm, indicating that charge-transfer transitions underlie the Soret absorption band.

(f) Resonance Interaction between the O-O Stretch and Porphyrin Ring Vibrations in the Dioxygen Complexes of Co "Picket Fence" Porphyrin and Co Mesoporphyrin IX-Substituted Myoglobin (with Mackin and Tsubaki, J. Am. Chem. Soc., in press).

"Resonance interaction" refers to the vibrational perturbation that occurs between two fundamental modes, whereas "Fermi resonance" is reserved for those cases where an overtone or a combination tone interacts with a fundamental. In the Co "picket fence" complex, the $\nu(^{16}O-^{16}O)$ at 1154 cm^{-1} shifts to 1079 cm^{-1} in the $^{18}O_2$ complex and undergoes resonance interaction with a 1079 cm^{-1} porphyrin mode. Also, a second ν(O-O) was observed in the $^{18}O_2$ complex at ~1108 cm^{-1}, which is also accidentally degenerate with a porphyrin ring mode, resulting in two new lines at 1098 and 1115 cm^{-1}. In the Co(II)(meso)myoglobin, the $\nu(^{16}O-^{16}O)$ at ~1125 cm^{-1} undergoes resonance interaction with the 1132 cm^{-1} porphyrin ring mode and two lines appear at 1104 cm^{-1} and 1137 cm^{-1}. The $\nu(^{18}O-^{18}O)$ at 1680 cm^{-1} is unperturbed. Ths observation of two ν(O-O) frequencies in the Co "picket fence" complex is attributed to two conformations of the N-methylimidazole plane relative to the Co-O-O plane. On the other hand, the oxy Co mesoporphyrin-IX-substituted myoglobin exhibits only one ν(O-O) frequency, indicative of one allowed conformer.

The ν(Co-O) stretching frequency was found at 516 cm^{-1} and 540 cm^{-1} in the "picket fence" complex and cobalt myoglobin, respectively. The 24 cm^{-1} difference between the two is explained in terms of possible difference in the Co-O-O angle resulting in weaker overlap between the orbitals involved in the $\sigma(d_{z^2})/\pi_g^*$) bond.

(g) Conformational Transitions and Vibronic Couplings in Acid Ferricytochrome c (with Lanier and Felton, Biochemistry, 18, 1656 (1979)).

Resonance Raman spectral changes in ferricytochrome c as a function of pH between 6.7 and 1.0 are reported and the structural implication is given in terms of "core-expansion" model advanced by us in 1975 (JACS, 97, 2517). The data are interpreted as indicating that the iron in high-spin acid ferricytochrome c (pH 2.0) with two water molecules as axial ligands lies in the plane of the porphyrin ring. At pH 1.0 there is a different high-spin form of cytochrome c which has an estimated iron out-of-plane distance of ~0.46A. Excitation at ~620 nm in acid

cytochrome c (pH 2.0) enhances only three depolarized vibrations at 1623, 1555, and 764 cm^{-1}. Marked enhancement of depolarized mode relative to polarized and anomalously polarized modes is attributed to the vibronic coupling between porphyrin $\pi \rightarrow \pi^*$ and porphyrin $\pi \rightarrow$ iron (d_π) charge-transfer states.

(h) Interaction of Divalent Metal Ions with Adenine Moiety of Adenosine 5'-Triphosphate (with Lanier, J. Biol. Chem., 254, 5882 (1979)).

Raman spectra of ATP at various pH values are affected by addition of equimolar solution of divalent metal ions such as Ca^{2+}, Mg^{2+}, Co^{2+}, Cu^{2+}, and Hg^{2+}. The changes in frequency and intensity have been used to construct models describing the nature of metal-adenine and metal-triphosphate interactions under different conditions. The metal ions are found to coordinate the triphosphate group in the entire pH range studied (pH 3 to 12). Calcium(II) and magnesium(II) interact strongly with the phosphate moiety at neutral pH, although a weak interaction with with ring occur at low pH values. Around neutrality, several Raman spectral changes are observed to implicate the interaction of cobalt(II) ion with five-membered ring of the adenine. At least six different Cu(II)-ATP species are identified between pH 3 and 12. At pH ~7.0 Raman data are explained better by Cu(II) interacting with N7 simultaneously with amino group of the adenine ring. However, a Cu(II) binding to N3 at pH 10 to 11 is indicated by the enhancement of the 760 and 1360 cm^{-1} vibrations.

(i) Resonance Raman Studies of Co(III)-ATP Complexes (with Lanier and Werber, J. Raman Spectrosc., 11, 150 (1981)).

Interactions of cobalt(III) ion with ATP in the presence of 1,10-phenanthroline and superoxide were studied by laser Raman spectroscopy. Striking intensity enhancement of two adenine ring vibrations at 735 and 1430 cm^{-1} was observed upon formation of the complex Co(III)-(phen)-ATP-$O_2^{\cdot -}$. The effect could be abolished by addition of cyanide ion to replace the superoxide ion. The spectral features were interpreted as indicating a direct binding of Co(III) to the C_6-NH_2 group of adenine. The interaction between Co(III) and 1,10-phenanthroline was manifested in the frequency and intensity changes near 436 and 1064 cm^{-1}. Raman intensities at 734, 1312, 1055 and 1430 cm^{-1} were measured as a function of laser exciting wavelength. The gradual changes in Raman excitation profiles of ATP lines from 647.1 nm to 457.9 nm indicated the pre-resonance effect with a charge transfer transition between adenine and Co(III), which may lie between 300 and 400 nm as evidenced by the more dramatic enhancement of these lines with 406.7 nm excitation.

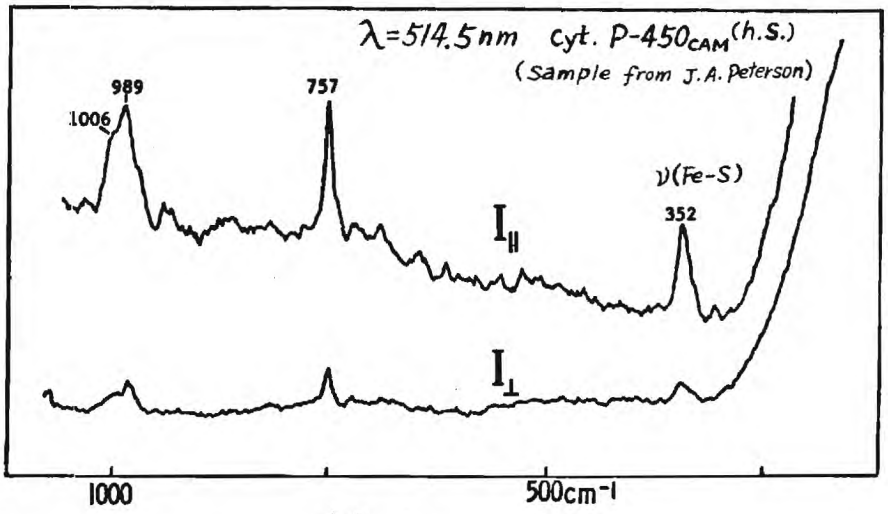


Fig. 4

(j) Two charge-transfer transitions, responsible for the resonance Raman enhancement of ν(Fe-S) stretch in cytochrome P-450 systems, have been located at ~550 nm and 340 nm. The ν(Fe-S) stretch at 352 cm^{-1} (Figure 4) first suggested by Felton and Yu (1978), was recently confirmed by Champion et al. (1982) using isotopically labelled samples of the oxidized cytochrome $P\text{-}450_{cam}$ substrate complex. Recent studies of cytochrome $P\text{-}450_{scc}$ (collaboration with Prof. J. David Lambeth of Emory University) also identified the existence of such a ν(Fe-S) stretch when excited at 351.1 nm.

(k) Preliminary studies on Fe(II)(TpivPP)(Pyridine)(CO) and Fe(II)(TpivPP)(THF)(CO) indicate that the ν(Fe-CO) stretch (a measure of the Fe-C bond strength) is very sensitive to the bonding between iron and trans ligand. For Fe(II)(TpivPP)(Pyridine)(CO) the iron-carbon bond length is ~1.77Å (Peng and Ibers, 1976) with its ν(Fe-CO) stretching frequency at 489 cm^{-1}; for Fe(II)(TpivPP)(THF)(CO) the iron-carbon bond length is ~1.71Å (Scheidt et al., 1981) with its ν(Fe-CO) stretching frequency at ~523 cm^{-1}. With a high precision Raman difference spectroscopy technique (detectable frequency difference ~0.1 cm^{-1}) (Rousseau, 1981), a bond length change as small as 0.0002Å can be detected by resonance Raman spectroscopy. (Kerr, Mackin and Yu, unpublished).

(l) Preliminary studies on Chang's strapped and unstrapped Fe(II)porphyrin (N-MeIm)(CO) complexes reveal interesting behavior of the δ(Fe-C-O) bending mode. The intensity of the δ(Fe-C-O) mode appears much stronger in the distorted CO than in the linear CO.

(m) Preliminary studies indicate that unlike penta-coordinate NO-heme complexes, the ν(Fe-CO) stretch in penta-coordinates heme-CO complexes such as Fe(II)(TpivPP)(CO) can be readily enhanced by excitation in the Soret region.

(n) Hori and Kitagawa (1980) reported the insensitivity of the ν(Fe-O_2) stretch to steric hindrance of the CH_3 group in the trans ligand, 2-MeIm or 1,2-Me_2Im. They located the ν(Fe-O_2) frequency at 568 cm^{-1} in both Fe(II)(TpivPP)(N-MeIm)(O_2) and Fe(II)(TpivPP)(1,2-Me_2Im)(O_2) in CH_2Cl_2. However, Walters et al. (1980) reported a 4 cm^{-1} difference between Fe(II)(TpivPP)(N-MeIm)(O_2)/CH_2Cl_2 (568 cm^{-1}) and Fe(II)(TpivPP)(1,2-M_2Im)(O_2)/CH_2Cl_2 (564 cm^{-1}). Recently, we studied Fe(II)(TpivPP)(N-MeIm)(O_2) and Fe(II)(TpivPP)(2-MeIm)(O_2) in benzene, indicating a 7 cm^{-1} difference in ν(Fe-O_2) stretching frequency between the two complexes. Further experiments are being carried out to settle the conflicting results between Kitagawa and Spiro's groups.